HISTOLOGIC CHANGES IN IRRADIATED SKIN, AFTER INGESTION OF 8-METHOXYPSORALEN*

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It is generally agreed that oral ingestion of 8-methoxypsoralen has some effect on skin pigmentation and on skin reaction to sunlight. Conflicting hypotheses and opinions have been advanced on the nature of these effects. S. W. Becker, Jr., reported that a stratum lucidum was formed in the skin after it was irradiated with either "an ultraviolet light", or with sunlight, two hours following ingestion of 20 mg. of methoxsalen (1).

This experiment was designed to answer the following questions. The answers derived are within the limits of the experiment. Methods of arriving at the answers given are described.

1) Q. Does ingestion of methoxsalen produce a stratum lucidum in ultraviolet irradiated skin, as reported by Becker?
   A. If the hyaline-appearing layers just above the stratum granulosum which appear similar to stratum lucidum are considered as stratum lucidum, as they were by Becker, the answer is, "yes". Previous reports by Miescher consider this as a thickening of the stratum corneum (2). It appears different from the remainder of the stratum corneum, so this becomes a question of semantics and awaits more selective differential stains.
2) Q. Does ingestion of methoxsalen produce a stratum lucidum or other change in non-irradiated normal skin?
   A. No.
3) Q. Does ingestion of methoxsalen produce a change in the unirradiated normal mucous membrane?
   A. No.
4) Q. What wave lengths (if any) produce changes synergistic with 8-methoxypsoralen?
   A. All wave lengths used produce some change. The most penetrating wave lengths, peaking at 2970 Å, produced the most intense quantitative changes.
5) Q. Do other (non-ultraviolet) stimuli to the skin (*e.g.*, non-specific irritation or X-radiation) produce histologic changes in synergy with ingested methoxsalen?
   A. Yes.
6) Q. What are these histologic changes?
   A. The most striking histologic change is the formation of a "stratum lucidum", or modified stratum corneum, just above the stratum granulosum. The other changes seem to be increased response to previously reported physiologic effects of radiant energy—*i.e.*, more thickening of the stratum corneum, more activity in the epidermis, more subepidermal edema and infiltrate. The increased adherence of the stratum corneum may be an effect specific for methoxsalen ingestion, and may cause the protective effects against sunburn previously reported.

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Presented at the Brook Lodge Invitational Symposium on the Psoralens, sponsored by The Upjohn Company, Kalamazoo, Michigan, March 27–28, 1958.

REVIEW

It is generally accepted that there are at least three pigment-forming stimuli in normal skin.

1) *Post-inflammatory pigmentation; injury to epidermal cells:* "H" substance, leukotaxine, or other, unknown, substances released by inflammatory changes in the skin, are followed by pigmentation of the epidermis.

2) *Ultraviolet → latent period → erythema → tan sequence: Ultraviolet irradiation in the maximum erythema wave lengths:* 2900 to 3200 Å wave lengths of ultraviolet approximate the absorption and action spectrum of tyrosine. When they strike the skin, they activate tyrosinase and catalyze the formation of dihydroxyphenylalanine (DOPA) from tyrosine. The phenylalanine compounds are then polymerized in a series of progressive chemical steps, to melanin.

3) *Non-specific skin darkening without latent period:* 3200–3900 Å (long ultraviolet) exposure produces immediate skin darkening, without

erythema or latent period. Some investigators feel that this is the same as the darkening produced by visible light and by heat, and that it is identical with the Meirowsky effect in cadavers. This is presumed due to an invisible "leuko" form of melanin, present in the skin from previous production, being oxidized to a visible brown.

EXPERIMENTAL DESIGN

Since all three procedures induce pigmentary changes in normal skin, investigation of each was attempted. It was also felt that a limitation of the wave lengths used in each experiment should be attempted, for more specific understanding of 8-methoxypsoralen action on pigment formation and skin reaction.

TECHNIC OF THE EXPERIMENT

Two volunteers (MCZ, SHM) were used as experimental subjects. Both were 37-year-old male brunettes, capable of good tanning response to sunlight stimulation.

Both subjects took 8-methoxypsoralen, 20 mg. daily, between breakfast and lunch. The test areas of the skin were irradiated, not less than three, nor more than six, hours later. This was continued for 14 consecutive days. Power punch three mm. biopsy specimens were taken from each area, as well as from control areas, and from the buccal mucous membrane, before starting (2/27/58), the seventh day (3/6/58), and the fourteenth day (3/13/58).

All sections were fixed in 10% formaldehyde solution, and at the conclusion of the experiment were all stained simultaneously in hematoxylin-eosin and iron hematoxylin.

SOURCES OF IRRADIATION

1) Universal X-ray Corp. Grenz X-ray machine: Factors—15 Kv; 12 ma; minimum wave length 0.8 Å; 3 cm. cone; TSD, 8 cm.; (HVL 0.034 mm. Al).

Exposure: 100 r daily.

2) "Cold" quartz exposure: (Dallon Mediquartz Lamp, two grids): Factors—The quartz grid emitted 385 microwatts cm.^{2} at 24 inches, the Corex glass grid 220/cm.^{2}. 90% of quartz grid radiation was at 2537 Å, 8% between 2600 Å, and 3000 Å, and 2% above 3000 Å. The Corex grid emitted 64% at 2537 Å, 33% 2600 Å–3000 Å, 3% above 3000 Å.

Exposure: 30 seconds daily, at a six inch distance.

3) *"Hot" quartz. (Air-cooled Kromayer (Hanovia) Mercury Arc Lamp):* Factors: At contact, produced minimum perceptible forearm flexure erythema in three seconds. Emission: 9% at 2537 Å. Other principal wave lengths: 2482, 2652, 2700, 2752, 2803, 2894, 2967, 3025, 3130, 3341 and 3660. 20% of the energy in the ultraviolet spectrum was in other wave lengths. Heat stimulus was maximum tolerable at exposure used.

Exposure: Contact, 30 seconds daily.

4) Same as above, filtered through window glass to eliminate wave lengths shorter than 3200 Å.

Exposure: Contact, 70 seconds daily.

5) Modified mercury glow discharge lamps: Two Westinghouse 24" Ultraviolet Fluorescent "Sun Lamp" tubes, side by side, parallel, 4½ inches apart.

This lamp has a campaniform spectrum, 90% of its energy between 2800–3100 Å. It was considered the most physiologic source of ultraviolet, since it had a continuous spectrum of wave lengths found in sunlight.

Exposure: 30 seconds daily, at a four inch distance.

RESULTS

I. Macroscopic

The areas of skin exposed to "cold" quartz, "hot" quartz, and modified mercury glow discharge lamps developed mild erythema and scaling from the second day on. The areas exposed to "hot" quartz filtered through window glass developed mild pigmentation and fine, branny scaling after one week of exposure. Areas exposed to Grenz radiation developed mild pigmentation and inflammation after ten days. This persisted for several weeks after the experiment was concluded. The scale was unusually tenacious in all areas.

II. Unirradiated areas—microscopic control

No significant difference was noted between specimens taken from skin and mucous membrane before the experiment was begun, and specimens taken from unirradiated areas of skin and mucous membrane after seven days and 14 days of methoxsalen ingestion.

III. Irradiated areas—microscopic

1) *Grenz radiation.* 3/6/58: Slight thickening of epidermis, with increased cellular activity in

stratum spinosum. Diffuse, mild epidermal edema and mild perivascular infiltrate, upper cutis.

3/13/58: Stratum corneum and stratum granulosum slightly thickened, with fine pigment granules present in the lower half of the stratum spinosum. The stratum corneum was more adherent, thicker and denser, than in the earlier sections.

2) *"Cold" quartz.* 3/6/58: Slight thickening of epiderm, with increase in cellular activity in the stratum spinosum, and superficial perivascular infiltrate in the cutis.

3/13/58: Densely adherent, thickened stratum corneum with trabeculated laminae at surface. The stratum corneum appeared homogeneous throughout. There was no evidence of a change in staining suggestive of stratum lucidum in these sections.

3) *"Hot" quartz.* 3/6/58: Epidermis shows diffuse thickening, with adherent stratum corneum alternating with small areas of parakeratosis. Pigment granules present throughout epiderm, from the top of stratum corneum down.

3/13/58: More pronounced thickening of the stratum corneum, plus mild edema and fragmentation of connective tissue just under the epidermis. Pigment granules were less evident than in the sections of 3/6.

4) *"Hot" quartz plus glass filtration.* 3/6/58: Stratum corneum thickened. Pigment in the supranuclear caps over the basal cells was slightly increased. The other layers of epidermis were not thickened, and did not show increased activity.

3/13/58: Stratum corneum showed moderate thickening, and an increase in adherence. The remainder of the epidermis showed no significant change, as compared to control sections.

5) *Fluorescent ultraviolet.* 3/6/58: Slight increase in thickness of the stratum corneum, which was densely adherent and showed scattered patches of superficial parakeratosis. There was also mild, diffuse thickening of all layers of the epidermis and mild edema present in all layers of the epidermis.

3/13/58: More pronounced diffuse edema of epidermis, the marked thickening of all epidermal layers. The densely adherent stratum corneum was greatly thickened and showed mild follicular plugging. There was pigment present throughout the lower half of the epidermis. There were scattered areas of hyaline appearing, homogenous bands just above the stratum granulosum, one to three squamous cell layers thick. These appeared similar to stratum lucidum as seen in normal sections of palms and soles.

CONCLUSIONS

Ultraviolet exposure to skin of subjects ingesting 8-methoxypsoralen results in increased pigmentation and increased thickening of the epidermis, especially the stratum corneum. This stratum corneum thickening seems to result from an increased cohesion of squamous cells so that the outer layers of the stratum corneum scale off less readily. After heavy ultraviolet irradiation in the 2970 Å region, hyaline changes under the stratum corneum appear, similar to stratum lucidum on palms and soles. The protective shield effect resulting from this phenomenon against cumulative changes caused by sunlight damage to the skin seems substantiated.

REFERENCES

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